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**Motherless quail mothers display impaired maternal behaviour and
produce more fearful and less socially motivated offspring**

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Running title: Maternal deprivation in Quail

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Early maternal deprivation impairs the behavioural development of young individuals. Recently, strong differences between mothered and maternally-deprived chicks have been reported concerning their emotionality, sociality, and spatial skills. Here we investigated long-term and cross-generational impacts of maternal deprivation by comparing the characteristics of the non-reproductive and the maternal behaviour of 22 mothered and 22 non-mothered adult female Japanese quail (*Coturnix c. japonica*) and by comparing the behaviour of their respective fostered chicks. We reveal that non-brooded mothers were more fearful and less competent in spatial tasks and expressed impaired maternal care, characterized by more aggression towards chicks, higher activity rates and more abnormal pacing during the first days of the care period. Chicks' behaviour was clearly affected by maternal care inducing strong differences in their fearfulness and social motivation. Our results show both long-term and cross-generational impacts of early maternal deprivation in precocial birds.

Key-words: Fearfulness; Japanese quail; maternal behaviour; maternal deprivation; maternal effects; precocial bird; sociality; spatial skills.

41 The behavioural development of animals is strongly influenced by early post-natal living
42 conditions, particularly interactions with the first care-giver between birth and emancipation
43 (Champagne & Meaney, 2001; Fleming, O'Day, & Kraemer, 1999). During this period, a
44 mother's behaviour affects her offspring's neuronal and physiological development with often
45 long-term consequences on their social and emotional behaviour (Berman, Rasmussen, &
46 Suomi, 1997; Caldji, Francis, Sharma, & Plotsky, 2001; Francis & Meaney, 1999) and their
47 cognitive skills (Bredy, Grant, Champagne, & Meaney, 2003; Liu et al., 2000). These
48 influences can occur through various social learning processes (Holmes & Mateo, 1998) or
49 via more passive mechanisms through which mothers directly modify the neurophysiologic
50 development of offspring by tactile stimulations (Champagne & Curley, 2009). Maternal
51 deprivation paradigms have been widely used to investigate the influence of mothers on the
52 subsequent behaviour of their offspring (see Fleming et al., 2002). Early deprivation can be
53 either partial, caused by precocious weaning (see Latham & Mason, 2008; Livia Terranova &
54 Laviola, 1995) or repeated separations (Dettling, Feldon, & Pryce, 2002; Millstein, Ralph,
55 Yang, & Holmes, 2006), or complete (Albers, Timmermans, & Vossen, 1999a; Bertin &
56 Richard-Yris, 2005; Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Melo et al.,
57 2006). Early repeated separations of young mammals from their care-giver affect their
58 emotional behaviour (Ogawa et al., 1994; Wigger & Neumann, 1999), sociality (Livia
59 Terranova & Laviola, 1995; Seay & Harlow, 1965; Tsuda & Ogawa, 2012), sexual behaviour
60 (Rhees, Lephart, & Eliason, 2001) and cognitive skills (Aisa, Tordera, Lasheras, Del Rio, &
61 Ramirez, 2008; Aisa, Tordera, Lasheras, Del Río, & Ramírez, 2007; Garner, Wood, Pantelis,
62 & van den Buuse, 2007).

Complete maternal deprivation of mammals has been comparatively less used. Nevertheless, several studies report that subsequent adult behaviour can be strongly impacted by maternal deprivation, such as reproduction (see Fleming et al., 2002; Gonzalez et al., 2001).

Most specifically, early social environment has strong effects on the quality and intensity of rodents' and primates' parenting behaviour expressed in adulthood: maternal deprivation impairs the expression of maternal behaviour. Harlow's famous studies showed that most of the macaque "motherless mothers" either appeared ignorant or were abusive to their infant (Arling & Harlow, 1967; Harlow, Harlow, Dodsworth, & Arling, 1966; Harlow & Suomi, 1971). Similarly, only 14% of the female chimpanzees reared by humans became competent mothers (Brent, Williams-Blangero, & Stone, 1996) and maternal deprivation led to impoverishment of rats' maternal behaviour (see A. S. Fleming et al., 2002; Gonzalez et al., 2001; Melo et al., 2006) even towards fostered pups (Palombo, Nowoslawski, & Fleming, 2010) confirming the direct effects of maternal deprivation on maternal care.

Without entirely reconsidering these results, several authors pointed out that mammals do not offer the possibility to measure rigorously the impact of complete maternal deprivation. Indeed, some care must be artificially provided by human interventions and the characteristics of this artificial care is likely to influence pups' development (Fleming et al., 2002). Comparatively, maternally deprived precocial birds can be reared correctly with human intervention limited to providing heat, and consequently they constitute perfect models to investigate the influences of complete maternal deprivation. Complete deprivation procedures have shown that fearfulness and social motivation (Bertin & Richard-Yris, 2005; Fält, 1978; Perré, Wauters, & Richard-Yris, 2002; Roden & Wechsler, 1998; Shimmura et al., 2010), spatial skills (De Margerie et al., 2012) and rhythmicity (Formanek, Richard-Yris, Houdelier, & Lumineau, 2009; Wauters, Perré, Bizeray, Leterrier, & Richard-Yris, 2002) differ strongly

between artificially reared and mothered chicks. Nevertheless, these differences have been evidenced only during the first weeks after hatching and comparatively far less is known about long-term consequences of maternal deprivation on precocial birds. The influence of maternal deprivation on the expression of precocial birds' maternal care remains comparatively unknown whereas other aspects of their reproduction behaviour are known to be affected by early experience (Immelman, 1972).

This study aimed to evaluate the long-term influences of total maternal deprivation on adult female precocial birds' emotional reactivity, sociality and spatial skills and on the way they subsequently care for their own chicks. Our model is Japanese quail (*Coturnix coturnix japonica*), a domestic precocial bird. Mothers are the only care givers in this species, and care lasts only for a short 11-day period. We first evaluated the behavioural characteristics of mothered and non-mothered females using well-established ethological tests (Bertin & Richard-Yris, 2004; Forkman, Boissy, Meunier-Salaün, Canali, & Jones, 2007; Formanek, Houdelier, Lumineau, Bertin, & Richard-Yris, 2008). We then induced maternal behaviour in these females and evaluated the maternal care expressed by mothered and non-mothered females. As differences in maternal behaviour are known to influence the behavioural development of Japanese quail offspring (Pittet, Coignard, Houdelier, Richard-Yris, & Lumineau, 2012), our last goal was to determine whether these potential maternal care differences impacted the way chicks subsequently behaved by comparing the behavioural characteristics of chicks fostered by females that developed either with or without a mothering hen. We hypothesized that, as for mammals, effects of maternal deprivation would still be observable in adult precocial birds, that their maternal behaviour would be impaired by this early deprivation and that this would subsequently affect chicks' behaviour.

Methods

Ethic Statement

All experiments were approved by the departmental direction of veterinary services (Ille-et-Vilaine, France, permit number 005283) and were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Our breeding procedure and our ethological tests were approved by the regional ethics committee (agreement number: R-2011-SLU-02).

Animals and Housing

All the subjects were from a broiler line and were provided by an industrial farm (Les Cailles de Chanteloup, Ille et Villaine, France). Food was available *ad libitum* and conditions were LD (light/dark) 12/12 and $20 \pm 1^\circ\text{C}$.

Grandmothers: 8-week-old adult females ($n=22$) were acquired by the laboratory, individually marked by a numbered ring on a wing and placed individually in wire mesh breeding cages (51x40x35cm) with a drinker and a feeder. They were left in their cage for 3 weeks before being given chicks for adoption, to become habituated to their environmental conditions. The breeding room contained 44 cages and the females were distributed so that two females were never in neighbouring cages. The other cages were equipped with a heater ($38 \pm 1^\circ\text{C}$) for rearing non-brooded chicks.

Mothers: They were 176 chicks that had been either adopted by “grandmothers” (brooded) or artificially reared (non-brooded). Male and female chicks were acquired by the laboratory when they were 1 day old. They were immediately placed in groups of 44 in 4 large plastic cages (98x35x42) equipped with a feeder, a drinker and a heater ($38 \pm 1^\circ\text{C}$). In the evening of their arrival they were divided into two groups: 22 sets of 4 chicks (brooded chicks) were

given to the 22 “grandmothers” and 22 sets of 4 chicks (non-brooded chicks) were placed in similar cages equipped with a heater. During this first breeding period, although cages with mothers needed more care interventions, we systematically intervened in all the cages to avoid differences between B and NB related to interactions with humans.

Adoptive mothers and warming lamps were removed when chicks were 11 days old, when they disperse naturally (Orcutt & Orcutt, 1976). Sex was determined when they were 3 weeks old and one female was chosen randomly from each cage, individually marked with a numbered ring on a wing and left in her breeding cage until she was 3 months old. When a brood did not include a female (5 broods), we took a female from another brood that included two or more females. Brooded females are noted B (n=22) and females that developed without mothers are noted NB (n=22). Emotional reactivity, sociality and spatial abilities of B and NB were assessed using several well-established poultry behavioural tests during the three weeks before they adopted chicks.

Chicks: 200 one-day-old newly acquired chicks were placed in groups of 40 in 5 large plastic cages (98x35x42) equipped with a feeder, a drinker and a heater ($38\pm 1^{\circ}\text{C}$). In the evening, 176 of these chicks were randomly selected to be adopted either by B (22 sets of 4 chicks: B-c) or by NB (22 sets of 4 chicks: NB-c). After they were separated from their mothers, the emotional reactivity, sociality and spatial skills of B-c and NB-c were evaluated.

Fostering procedure and observation of maternal Behaviour

Fostering procedure

The same fostering procedure was used to induce maternal behaviour in both grandmothers and mothers.

Maternal behaviour was induced using the induction procedure described by Richard-Yris (1994). At the beginning of the dark phase (20:00), 4 one-day old chicks were placed gently underneath each female who had been shut up in her nest box (18x18x18cm) one hour before. Boxes were shut up again for the whole night (12 hours) during which the chicks' vocal and physical solicitations induced rapid expression of maternal behaviour by the adult females. The next morning, all the boxes were opened and removed from the cages at 08:00. During the first day that the mothers spent with this first brood, their maternal behaviour was monitored and females that did not express any warming behaviour of the young chicks or that expressed aggression resulting in injuries of chicks were excluded from the experiment: none of the 22 females had to be excluded from the initial breeding period (grandmothers breeding mothers), but 4 B and 2 NB were excluded from the second breeding (B and NB breeding respectively B-c and NB-c) period.

Chicks that showed signs of hypothermia (motionless, eyes closed and trembling) when leaving the boxes or later during the breeding period were discarded from the experiment. They were immediately placed under heaters where they recovered swiftly. These chicks were replaced by chicks of the same age, identified by a leg ring, so that they were not tested later. At the end of the breeding period, 60 NB-c and 57 B-c had spent the whole breeding period with their mother. Numbers of chicks replaced did not differ significantly between NB and B (NB: 1.15 ± 0.33 , B: 0.67 ± 0.21 , $U=206$, $p=0.4$) and as many NB as B broods involved at least one replacement during the breeding period (NB: 13/20, B: 11/18, $\chi^2=0.06$, $p=0.8$).

Mothers were removed from the cages on post-hatching day (PHD) 11. Chicks then remained with their siblings for two more weeks during which the fearfulness, the social motivation and the spatial skills of two chicks from each cage were evaluated (when two chicks that spent the whole breeding period with the mother were available: n NB-c = 32; B-c = 33). As

morphological sexual dimorphism appears only at 3 weeks (Mills, Crawford, Domjan, & Faure, 1997), chicks were chosen randomly, but sex ratios did not differ significantly between B-c and NB-c (sex ratios: B-c=0.88, NB-c=0.89; $\chi^2=0.00$, df = 1, p=0.99). During this test period, chicks were weighed when they were 14 and 28 days old.

Observations of maternal behaviour

Maternal behaviour was recorded on PHD 3, 5, 7 and 9. During the breeding period when B and NB females adopted chicks, we evaluated maternal behaviour using instantaneous scan sampling to establish mothers' time-budgets and, simultaneously, focal animal sampling to note complete behavioural sequences (see below for details). The observer stood behind a one-way mirror.

Instantaneous scan sampling: Each day we recorded 60 scans at 5-minute intervals: 30 scans in the morning and 30 in the afternoon. Each scan recorded whether the mother was warming chicks and if she was, we recorded her posture and how many chicks were being warmed. We also recorded the mother's activity and the distance between each chick and its mother. An index of distance was calculated as the average score of all chicks that were not being warmed. The five classes of distances and associated scores were: on opposite sides of the cage (1), more than half a cage away (0.75), less than half a cage away (0.5), one chick length away (0.25) and in contact (0). The higher the index was, the further from the mother the chicks were when not being warmed. We also recorded whether chicks were warming one another. Data were sampled using an ipod Touch (Apple©) and the application "scan sampling" (Vincent Richard ©).

Focal sampling: Each cage was observed for two 5-minute sessions when the mother's entire behavioural sequence was recorded. The experimenter noted the frequency of interactive

203 behaviours (aggression: the mother attacked chicks while producing threat vocalizations followed
204 by the chick moving away, pecking: the mother pecked at the chick with her beak closed, trampling
205 chicks and contact breaks) and non-interactive behaviours (locomotion, exploration,
206 observation and maintenance).

207 Separation test

208 To assess the strength of the bond between mothers and chicks, we removed chicks from each
209 cage for 5 minutes on PHD 6 and placed them in similar cages without their mother. Mothers'
210 reactions to this separation were observed behind a one-way mirror and the observer recorded
211 vocalizations and latencies to resume comfort behaviours (eating and resting). At the same
212 time, the reactions of 2 chicks a cage were videotaped to assess latency and frequency of their
213 distress calls and steps.

214 **Behavioural characteristics of foster mothers and chicks**

215 Procedures assessing fearfulness

216 Behavioural tests assessed the levels of fearfulness of both B and NB mothers before they
217 adopted chicks and of their chicks after separation. As fearfulness is a multidimensional trait
218 (Mignon-Grasteau et al., 2003) that cannot be estimated by a single procedure, several tests
219 presenting different environmental conditions and various fear-inducing stimuli were realized.
220 We investigated fearfulness using the procedures described below to assess shyness,
221 neophobia and reactions to humans.

222 All observations except reactions to humans were recorded behind a one-way mirror.

223 *Emergence test:* This test followed a protocol similar to that described by Jones et al. (1991).
224 Quail were individually transported in a wooden box (18x18x18cm) with a removable wall.
225 This box was placed against the apparatus: a large and well-lit wooden box (62x60x33cm)

with wood-shavings covering the floor and an observation window. The transport box was kept closed for 1 minute before the door was opened to allow access to the apparatus. The experimenter noted the latency between raising of the door and the emergence of the subject's head out of the box, and its total emergence.

Novel object test: This test assesses neophobia by the reactions subjects express in the presence of an unfamiliar object (R. B. Jones, 1996). Mothers were tested in their home cage, but chicks had to be socially isolated and were consequently tested in a polyhedral openfield (1m², h=60cm) after a 5-minute habituation. The novel stimulus was an unfamiliar plastic T-shaped object. During a 10-minute focal sampling the experimenter recorded latency to approach the object, frequency of locomotion (walks, runs) and frequency of fear behaviours expressed towards the novel object including escape, withdrawal (slowly walk away from the object keeping it in sight), jumps, fear postures (crouching) and freezing (Jones, 1996; Mills et al., 1997). The experimenter also recorded the frequency of defecation, observations and explorations of the object (pecking at the object), of the apparatus (pecking at a wall or at the floor). Scan samples, at 10-second intervals, recorded at the same time the position of the subject in relation to the object. During observations of mothers, the cages were virtually divided into two zones and the experimenter noted whether the female was in the half of the cage containing the object or not. During observations of chicks, the openfield was divided into three equal zones and the experimenter noted whether the chick was in the object zone, in a middle zone or in the zone opposite to the object.

Human observer test: This test assesses reactions to humans (Jones, 1993). Subjects were tested in their familiar environment. The experimenter, using instantaneous scan sampling, passed in front of each cage at 5-minute intervals recording a total of 32 scans for each cage. Each time he passed in front of a cage, he stopped for few seconds and recorded the

instantaneous activity of all subjects: fear reactions (subject interrupts its ongoing activity and moves away from the observer), observation of the observer, explorations, feeding, resting or maintenance behaviours (resting, self-preening).

Procedures to assess sociality

Inter-individual distances in home-cage: When they were 21 days old, inter-individual distances between each chick and its nearest conspecific were recorded by 32 scans of each cage, made at 4-minute intervals. We used the same distance classes and scores as those used to describe distances between mothers and chicks during the brooding period.

Runway test: This test is an adaptation of the treadmill test (Mills & Faure, 1990) that evaluates subjects' motivation to reach a social stimulus (Formanek et al., 2008). The apparatus is a 100cm-long wire-netting tunnel. Test subjects were transported individually in a wooden box (18x18x18cm), which was then placed at the tunnel entrance. At the other end of the tunnel was a cage (20x35x20cm) containing three unfamiliar conspecifics of the same age as the tested individual, representing a social stimulus. The corridor was divided into four zones: the closest zone to the social stimulus, "1 bird long" (zone P) and 3 equal 32cm-long zones called, from the entrance to zone P: zones A (beginning of the tunnel), B (middle) and C (end of the tunnel). One minute after the transport box had been put in place, the door was opened and the subject was observed for 5 minutes. The experimenter noted latency to emerge completely from the box, to reach zone P, number of crossed zones and time spent in each zone. An index of sociality was calculated using the following formula:

Index of sociality = Time (s) in zone P + 0.66*time in zone C + 0.33*time in zone B.

The higher the index is, the closer the individual remained to the social stimulus. The experimenter also recorded latency to emit a distress call, frequency of distress calls,

exploration of the cage containing conspecifics (number of soft pecking against the conspecifics' cage), aggressive behaviour (number of violent pecking against the conspecific's cage, associated with threat vocalizations), fear postures, and jumps.

Reaction to a stuffed conspecific: Only adult females were tested to assess their reactions to a model adult Japanese quail. The social stimulus was a stuffed female quail. Test subjects were first placed in the centre of a plastic arena (Ø120x60cm) with a linoleum floor, for a 5 minutes habituation period. Then the light was switched off for one minute when the lure was placed in the centre of the apparatus. When the light had been switched on again they were observed for a 5-minute focal sequence with the lure and, after a one minute without light, for another 5-minute focal sequence without the lure. During each sequence, the experimenter recorded latency of first distress call, number of distress calls, latency of first step, number of steps and frequencies of observation, exploration and maintenance activities. When exposed to the lure, latencies to approach and to contact the stuffed female were also recorded.

Spatial skills test

Detour task: The apparatus was a cross between Zucca & Sovrano's (2008) and Zucca, Antonelli & Vallortigara's (2005) detour apparatuses (Fig. 1). Test mothers and chicks were food deprived for 12h by presenting food for only 2 minutes at middle of the restriction period but water was available continuously. The apparatus was a rectangular arena with an obstacle through which a feeder placed just behind it could be seen. Test subjects were placed in the dark in front of the obstacle and had to go round it to reach the feeder. The experimenter noted latency to take first step, to get round the obstacle, to reach the feeder, which side of the obstacle the subject went and all the subject's behavioural activities (vocalizations, locomotion, jumps, observations, fear posture and maintenance).

Figure 1

Statistical analyses

As most of our data were not normally distributed, we used non-parametric tests to compare NB's and B's, as well as, NB-c's and B-c's behavioural data, separately per each behavioural variable. Mann-Whitney tests were performed to compare frequencies, latencies and proportions of time (spent in a particular area or performing a particular behaviour) between sets, and Chi-square tests were performed to compare proportions of quail of each set that expressed or did not express a behaviour.

For bivariate data (repeated measurements), we computed ANOVAs on repeated measurements after checking response variable and residual normalities by Kolmogorov-Smirnov tests and the homogeneity of variances by Mauchly's sphericity test. Data analyses were computed using Statistica® and XLStat®.

Results

Long-term effects of mother deprivation on adult behaviour

Effects on emotional reactivity

During the emergence test, NB put their head out of the shelter earlier than did B (NB: 5.68 ± 2.09 s, B: 20.81 ± 8.10 s; Mann-Whitney U-test: $U = 132$, $P = 0.01$), but latencies to emerge completely from the shelter did not differ between the two sets of females ($P > 0.05$). When facing a novel object, latencies to approach and to explore the object did not differ significantly between NB and B ($P > 0.05$). Nevertheless, NB moved more frequently (NB: 35.0 ± 2.7 , B: 26.7 ± 3.0 ; $U = 153$, $P = 0.04$), avoided the object more ($U = 120$, $P = 0.004$; Fig. 2a) and defecated more than did B (NB: 0.77 ± 0.11 , B: 0.45 ± 0.13 ; $U = 168.5$, $P =$

0.05). The frequencies of other behaviours did not differ significantly between B and NB ($P > 0.05$). More B than NB spent a significantly higher proportions of time near the object (NB: 8/18, B: 13/17; $\chi^2_1 = 3.73$, $P = 0.05$). NB females expressed more fear postures in reaction to humans ($U = 159$, $P = 0.04$; Fig. 2b). The proportions of scans spent in other activities did not differ significantly between B and NB ($P > 0.05$).

Figure 2

Effects on sociality

NB took longer to approach the lure than did B (NB: 79.41 ± 13.47 s, B: 48.18 ± 13.05 s, $U = 159$, $P = 0.05$). After removal of the lure, NB took longer to take their first step (NB: 85.77 ± 13.03 s, B: 27.59 ± 9.75 s; $U = 111.5$, $P = 0.002$) and tended to take less steps (NB: 25.5 ± 7.68 , B: 38 ± 8.74 ; $U = 162$, $P = 0.06$). The other behavioural traits measured in this test did not reveal significant differences between NB and B ($P > 0.05$). The runway test revealed no significant differences between females' behavioural expressions or the times they spent in the apparatus' different zones ($P > 0.05$).

Effects on spatial skills

Fewer NB than B were successful in the detour task (NB: 4/22, B: 13/22; $\chi^2_1 = 7.76$, $P = 0.005$). None of the other behavioural traits measured in this test, including latency to take first step and frequency of locomotor acts differed significantly ($P > 0.05$).

Effects of mother deprivation on maternal behaviour

Reaction to induction

The first interactions with chicks (aggressive behaviour, warming parameters) of females that were maternal after the induction procedure and the number of chicks showing signs of hypothermia during the first day following induction did not differ significantly between B and NB (Mann-Whitney U-test: $P > 0.05$).

Maternal traits

Brooding parameters (time spent warming chicks, number of chicks warmed and posture preferences), contact breaks or distance to chicks did not differ significantly between B and NB during the whole breeding period (Mann-Whitney; $P > 0.05$).

Nevertheless, aggressive behaviours towards chicks and time-budgets showed several differences between the two sets of females.

Indeed, NB were more aggressive towards chicks than B at the beginning of the brooding period (PHD3: NB: 2.15 ± 0.71 ; B: 0.39 ± 0.23 ; Mann-Whitney U test: $U = 119.5$, $P = 0.04$) and pecked them more on PHD 7 (NB: 1.15 ± 0.45 , B: 0.11 ± 0.08 ; $U = 132$, $P = 0.03$). Frequencies of chick trampling did not differ between B and NB females on any day of the breeding period ($P > 0.05$). NB's and B's time-budgets differed. NB spent more time active than B (see Fig. 3 for details), but their activity was not affected by chicks' age and we found no significant interaction between set of females and age of chicks (Fig. 3). More NB than B expressed stereotypic pacing (abnormally repeated flight attempts with exaggerated locomotion and pecking against the cage walls) on PHD3, PHD5 and tended to on PHD7 (Fig. 4), and NB spent higher proportions of time pacing on these days (PHD3: NB: $4.16 \pm 1.56\%$, B: $2.50 \pm 2.50\%$; Mann-Whitney U-test: $U = 96.5$, $P = 0.003$; PHD5: NB: $2.16 \pm 0.46\%$, B: $1.57 \pm 1.13\%$; $U = 112.5$, $P = 0.03$).

Figure 3

Figure 4

Reactions to separation

Reactions (latencies and frequencies of behaviours and vocalizations) to separation from chicks did not differ significantly between B and NB on PHD6 ($P > 0.05$). Similarly, reactions to separation from their mother did not differ significantly between NB-c and B-c ($P > 0.05$)

Chicks' growth and behaviour

Weights of NB-c and B-c did not differ significantly on PHD 14 or on PHD 28, but we found an interaction between age and set due to B-c's faster weight gain (Fig. 5).

Figure 5

Emotional reactivity

Emergence test data indicated that NB-c's mean latencies were more than twice as long as B-c's for both emergence of the head (NB-c: 18.5 ± 6.54 s, B-c: 5.53 ± 2.26 s; $U = 354.5$, $p = 0.005$) and full emergence (NB-c: 21.76 ± 6.78 s, B-c: 8.47 ± 3.34 s; $U = 307$, $P = 0.0007$). When exposed to the observer, NB-c spent less scans self-preening (NB: 0.76 ± 0.33 scans; B: 2.16 ± 0.63 scans; $U = 462$, $P = 0.04$). We could find no other significant differences between NB-c's and B-c's behavioural expressions during the emergence test and the reaction to the observer test ($P > 0.05$). NB-c's and B-c's behaviours did not differ significantly in the novel-object test ($P > 0.05$).

Sociality

Observations of NB-c in their cages indicated that they tended to be more frequently in parts of their cage opposite to their nearer conspecific (NB-c: $4.1 \pm 0.7\%$, B-c: $2.9 \pm 0.6\%$, $U = 490$, $P = 0.09$). Distance scores did not differ significantly between NB-c and B-c ($P > 0.05$).

Our distance index indicated that NB-c were further from the social stimulus in the runway test (NB: 160.9 ± 16.0 , B: 204.8 ± 16.1 , $U = 378$, $P = 0.034$). Moreover in this test, NB-c crossed more zones (NB-c: 17.8 ± 1.77 , B-c: 11.31 ± 1.25 ; $U = 321.5$, $P = 0.004$) and emitted distress calls earlier than did B-c (NB-c: 188 ± 21.27 s, B-c: 254.81 ± 15.43 s; $U = 392$, $P = 0.032$).

Spatial skills

As many NB-c as B-c were successful in the detour task (NB-c: 24/32, B-c: 24/33; $\chi^2_1 = 0.04$, $P > 0.05$) and the latencies of the successful subjects to reach to the feeder did not differ between the two sets (Mann-Whitney U-test: $P > 0.05$).

Discussion

This study evaluated the modifications induced by maternal deprivation on females' subsequent non-reproductive and maternal behaviour in adulthood. We found that maternally deprived females displayed higher fearfulness and impaired spatial skills. When maternal, these motherless mothers were more aggressive towards chicks and more active during the first half of the care period when they also expressed more stereotypic behaviours than did mothered females. When assessing consequences of these maternal care differences on chick development, we found strong differences between NB-c and B-c as NB-c's fearfulness was higher and their social motivation was lower.

Effects of maternal deprivation on adult behaviour

First we showed that maternal deprivation impacts the non-reproductive behaviour and spatial skills of females when adult. Although previous reports have shown differences between mothered and non-mothered precocial birds, these reports assessed their behaviour only during the first weeks after hatching and no conclusions could be drawn concerning the long-term influences of this early experience (Bertin & Richard-Yris, 2005; de Margerie et al., 2012; Formanek et al., 2009; Perré et al., 2002; Roden & Wechsler, 1998; Shimmura et al., 2010).

In the present study, NB were clearly more fearful than B, they were more neophobic in the presence of a novel object and more fearful in the presence of humans. These results are consistent with Roden's (1998) and Perré's (2002) reports concerning domestic chicks, but contradict reports concerning Japanese quail chicks (Bertin & Richard-Yris, 2005). This last inconsistency might be related to the age or sex of test subjects (adult females vs. mixed-sex chicks), but we think they result from the adoptive mothers. Indeed, the females Bertin & Richard-Yris (2005) used as adoptive mothers for the brooded chicks had been selected for a particular level of emotionality (Mills & Faure, 1991). As maternal emotional reactivity is transmitted to fostered chicks (Houdelier et al., 2011; Richard-Yris, Michel, & Bertin, 2005), using mothers from different lines can affect chicks in such a way that they appear either more or less fearful than non-brooded chicks.

Another reported consequence of maternal deprivation is impairment of development of sociality (Dettling et al., 2002; Livia Terranova & Laviola, 1995; Seay & Harlow, 1965; Tsuda & Ogawa, 2012). We found that NB reacted more fearfully to a stuffed conspecific than did B. This reaction could be considered either as a lesser social competence or as a neophobic reaction because they had never been exposed to an adult. The fact that the runway test results revealed no differences in proximity with unfamiliar conspecifics between the two

sets, led us to favour the second explanation. Non-brooded chicks were reported to behave less socially than chicks that developed with a mother (Bertin & Richard-Yris, 2005; Perré et al., 2002), but we did not find a similar difference when they were adult. We do not suggest that this result indicates no differences in sociality between B and NB, but rather that characteristics of this species are involved (Guyomarc'h & Saint-Jalme, 1986), as adult females display solitary phases whereas chicks are highly gregarious, making differences in sociality much harder to highlight in adults than in chicks.

NB's detour results suggest that their spatial skills have been impaired. Exploratory behaviour of these subjects was assessed when they were chicks (in mixed-sex flocks of 4 mothered or non-mothered chicks), and the exploratory skills of non-mothered chicks already showed a deficit (De Margerie et al., 2012) that seems to be still observable in adulthood. This impairment of spatial skills is consistent with the literature reporting the necessity of maternal stimulation to promote mammals' hippocampal synaptogenesis and spatial learning (Liu et al., 2000). Early maternal deprivation experiments highlighted delayed or impaired spatial learning (Aisa et al., 2007; Garner et al., 2007). As suggested by de Margerie (2012), NB's spatial skills could also be a by-product of their higher fearfulness as fear-related behaviour can inhibit exploration (Murphy, 1978), and NB behaved more fearfully in other tests. Nevertheless inhibition of NB's exploration is not supported by our results since latencies to move and frequencies of moving, freezing or flight attempts did not differ significantly between NB and B. We consequently consider that the impoverishment of spatial stimulations due to maternal deprivation during NB's early life induced their poorer spatial ability.

Effects of maternal deprivation on maternal behaviour

Our results indicate that the maternal behaviour of maternally deprived females was impaired, at least at the beginning of the breeding period. This impairment is characterized by more

aggression, more activity and more frequent pacing stereotypies. The negative impact of maternal deprivation has already been reported in mammals. The maternal behaviour of hand-reared female primates is greatly deficient. Brent (1996) reported that only 14% of hand-reared chimpanzee females were able to provide adequate care and ensure the survival of their infants. Maternally-deprived female primates also behave aggressively towards their offspring (Harlow & Suomi, 1971). Artificially reared rats retrieved fewer pups during a retrieval test and exhibited reduced pup licking and crouching behaviours (Gonzalez et al., 2001). Currently, we cannot draw any conclusion about a direct influence of early experience on aggression of chicks as this could also be the expression of NB's higher fearfulness. High levels of activity when facing stressful situations and expression of stereotypic behaviours are also a known particularity of maternally-deprived mammals (Gonzalez et al., 2001; Latham & Mason, 2008). Interestingly, differences between NB and B females were only measurable during the first half of the breeding period. Contrary to all expectations, primate motherless mothers' brutality or indifference finally decreases in response to infants' persistent solicitations (Harlow & Suomi, 1971). We suggest that, similarly, stimulation of their mother by chicks eventually induces NB mothers to express a maternal behaviour comparable to that of brooded females.

Nevertheless, maternal deprivation did not appear to modify several fundamental traits of maternal behaviour, including time spent warming, warming posture preference or the strength of bond with chicks (as reactions to separation from chicks did not differ between NB and B). This result could suggest low plasticity of these traits to experiential factors since they were not clearly influenced by the presence of a mother during the first weeks of the female's life or by the female's breeding experience (Pittet, Coignard, Houdelier, Richard-Yris, & Lumineau, in press). This hypothesis subsequently implies the existence of an important

individual determinant that could be highlighted by analysis of mothering styles in our species as reported for many mammals (P. Albers, Timmermans, & Vossen, 1999b; De Lathouwers & Van Elsacker, 2004; Dwyer & Lawrence, 2000; Hill, Greer, Solangi, & II, 2007; Maestripieri, 1994), but never investigated in birds. We can also consider that chicks' rearing conditions and particularly the fact that they were reared in social groups strongly influenced this result. Several studies dissociate the confused effects of early social isolation and maternal deprivation in mammals and show that deprived individuals reared with social peers had reduced social and maternal deficits (Melo et al., 2006). Similarly, social conditions may have limited the impact of maternal deprivation in our study. Another possibility is that the expression of precocial animals' maternal care is less sensitive to early maternal stimulations than that of altricial species. The maternal care of precocial guinea pigs reared in social isolation, with social partners or with a mother and social partners did not differ (P. Albers et al., 1999a; Stern & Hoffman, 1970).

Development of chicks brooded by NB and B mothers

The fact that our results indicate that the early rearing conditions of mothers did not influence chicks' survival rates appears logical as most of the fundamental maternal behaviour traits did not differ between B and NB mothers and our laboratory conditions were non-restrictive. We nevertheless found that chicks brooded by B mothers presented a higher weight gain after separation from their mothers. We could consider here that the slightly harsher conditions in which NB-c developed as their mothers were more aggressive and more active probably impaired their weight gains slightly from the first days although differences between NB-c and B-c became significant only a few weeks after separation. Another possibility is that slight maternal behaviour differences did not affect weight directly, but as NB-c were more reactive in several tests, the testing period could have been a more stressful for these chicks

and led to a lesser weight gain after separation from the mother since fearfulness and fear behaviour are negatively associated with growth (Jones, Satterlee, & Marks, 1997).

Ability to resolve the detour task did not differ between the two sets of chicks and chicks' success was higher than that of their mothers. Our results indicate that the presence of a mother during early development clearly influences the future ability to resolve spatial tasks. Results from mammals indicate that the behavior of mothers can affect the spatial behavior of their offspring (Albers, Timmermans, & Vossen, 2000). Thus we expected the differences between NB's and B's maternal behaviour to induce differences in chicks' detour abilities. Indeed, precocial chicks brooded by a hen tend to follow their mother and hence explore larger areas than non-brooded chicks (Wauters et al., 2002) and show greater ability to explore new environments (De Margerie et al., 2012). NB's higher frequencies of maternal aggression and pacing could have led NB-c to follow their mother less and to be, consequently, less stimulated to explore their cage. The absence of effects of these maternal behaviour differences on NB-c's and B-c's spatial abilities could be related to physical characteristics of their housing environment where distances are limited and individuals never lose sight of one another. We suggest that such maternal stimulations differences could have stronger impacts on chicks' later spatial abilities if the breeding period took place in a larger and structured environment.

We found that the emotional and social behaviour differed between chicks brooded by mothered and non-mothered females. This result is in accordance with our previous reports and confirms an influence of maternal care on chicks' subsequent behavioural development. Interestingly, we highlighted maternal behaviour differences between NB and B only during the first half of the care period, but nevertheless chicks' behaviour differed greatly between sets, suggesting that they were more sensitive during this early period. NB-c were more

fearful than B-c in both a novel environment and in the presence of humans, traits that clearly paralleled the differences observed between their mothers. NB-c were also less socially motivated, as they were further from both familiar and unfamiliar conspecifics. We did not find evidence of any differences between their mothers concerning their sociality when adult, but, as chicks, B and NB presented similar differences (Bertin & M. A. Richard-Yris, 2005). Altogether, these results describe a clear case of non-genomic transmission of behavioural characteristics from mothers to chicks, similar to that already reported for both emotionality (M. A. Richard-Yris *et al.*, 2005) and sociality (Formanek *et al.*, 2008) in quail.

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Figures and legends

Figure 1

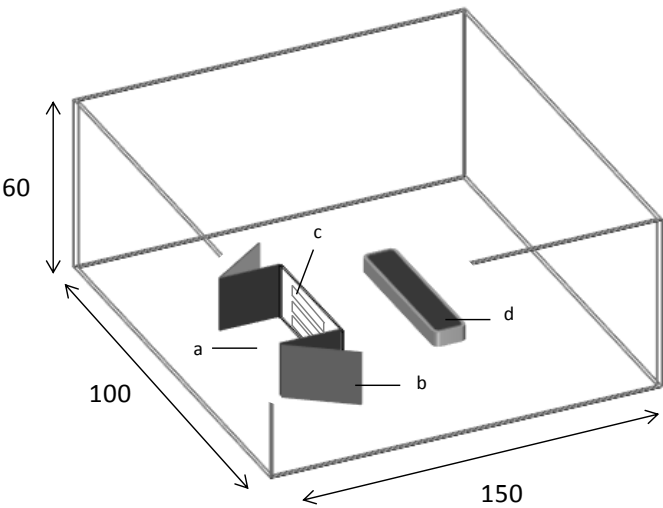
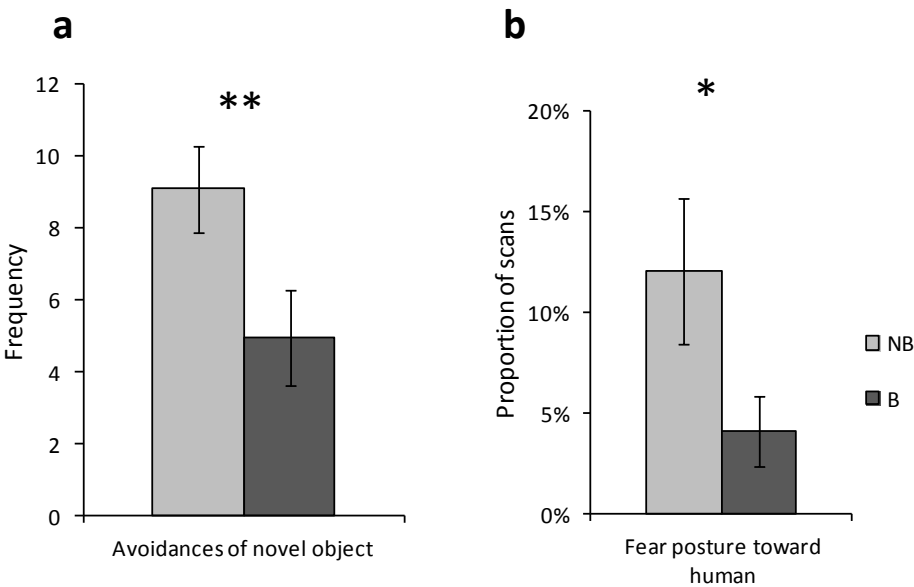
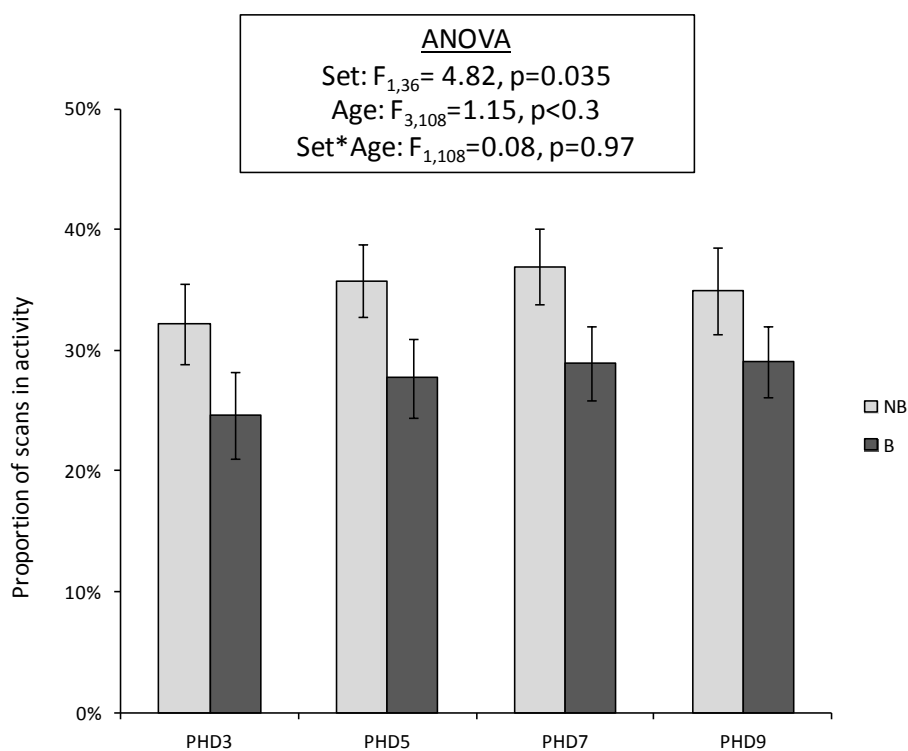


Figure 2

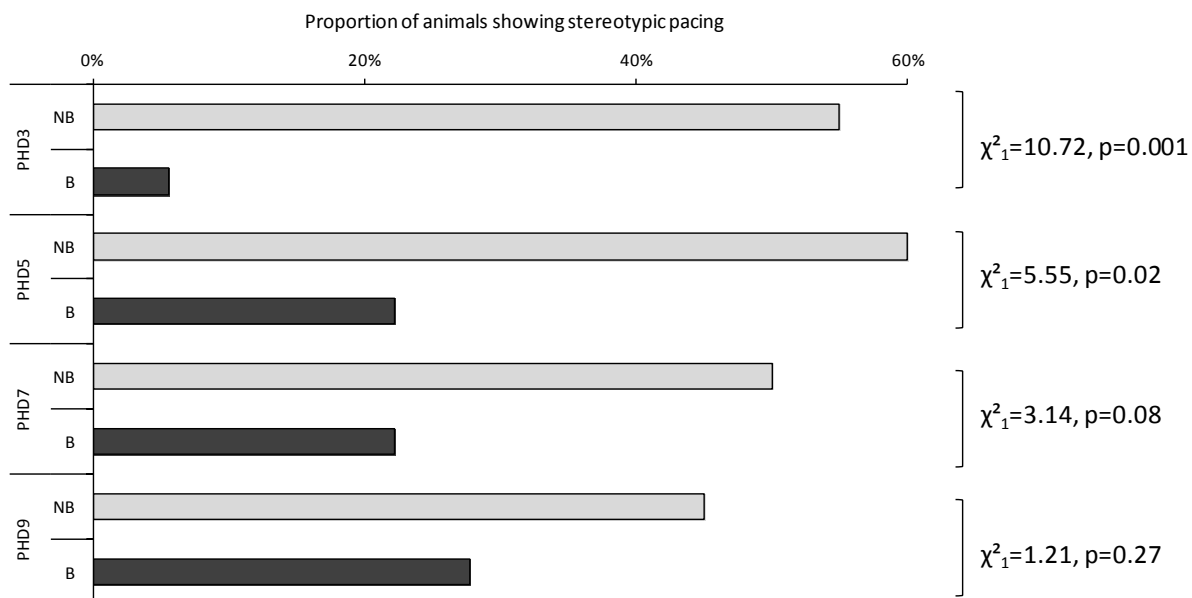


734 Figure 3



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736 Figure 4



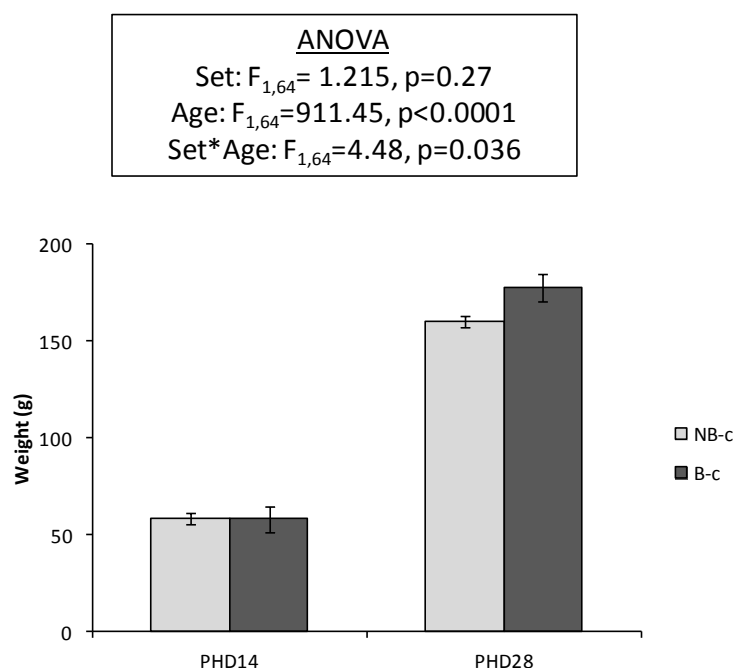
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741 Figure 5



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745 Figure 1. Schematic representation of the detour task with dimensions (in centimetres). a: start
746 location, b: obstacle, c: window, feeder.

747 Figure 2. a: Novel object test. Mean (\pm SEM) frequencies of avoidance of the novel object by
748 NB (grey bar) and B (black bar). b: Reaction to humans test. Mean (\pm SEM) proportions of
749 scans (in % of total number of scans) in fear postures in the presence of a human by NB (grey
750 bar) and B (black bar). Mann-Whitney U-test: ** $p<0.005$, * $p<0.05$. (nNB=22, nB=22).

751 Figure 3. Mothers' activity in relation to chick age. Mean (\pm SEM) proportions of scans (in %
752 of total scans) when B and NB were active (including locomotion, exploration, feeding,
753 stereotypic pacing, jumps, alert observations, pecking and aggression). PHD: Days of the
754 breeding period post hatching. Analysis of variance indicated that NB's (n=20) activity level
755 was higher than B's (n=18), but no effect of chicks' age, nor an interaction between set and
756 age of chicks. Grey bar: NB; black bar: B.

757 Figure 4. Mothers' stereotypic pacing in relation to chick age. Proportions of subjects that
758 expressed stereotypic pacing during the breeding period. Chi-square tests indicated that more
759 NB (n=20) than B (n=18) expressed stereotypic pacing at that beginning of the breeding
760 period (PHD3 and PHD5). Grey bar: NB; black bar: B.

761 Figure 5. Chicks' weights in relation to their age. Mean (\pm SEM) weight of NB-c and B-c on
762 PHD14 and PHD28. Analysis of variance indicated an effect of age and an interaction
763 between set and age. (nNB-c=32, nB-c=33). Grey bar: NB-c; black bar: B-c.